(9)

PLANT DEVELOPMENT WORKSHOP

SATURDAY, OCTOBER 31, 1981 9:00 A.M. - 4:30 P.M.

SCARBOROUGH COLLEGE

UNIVERSITY OF TORONTO

WEST HILL, ONTARIO

Everyone interested in Plant Development is invited to the 9th Plant Development Workshop for a day of presented papers, discussion, and socializing.

Preliminary schedule:

9:00 - 9:30	Coffee & Registration
9:30 - 12:00	Contributed papers
12:00 - 1:30	Lunch
1:30 - 4:00	Contributed papers
4:00	Refreshments

Graduate students in particular are encouraged to present their ideas and work in 15 minute talks. Please submit titles as soon as possible and brief abstracts by October 21.

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9th PLANT DEVELOPMENT WORKSHOP

Scarborough College, University of Toronto October 31, 1981

9:00-9:30 Registration, coffee and donuts, Room H-403B (Faculty Lounge)

9:30-10:45 First session, Room H214

1. Are cell proliferation and cell growth regulated by a single control system? D. Davidson, Biology Department, McMaster University.

2. Differences in mean doubling time in adjacent rows of cells. D. Davidson, Biology Department, McMaster University.

3. Asymmetry of mitosis: are the unequal divisions functionally significant? S.W. Armstrong and D. Davidson, Biology Department, McMaster University.

4. The ultrastructure of cell and nucleomorph division in the alga Cryptomonas L. McKerracher, York University.

10:45-11:05 Coffee, Room H-403B

11:05-12:20 Second session, Room H214

5. Studies on possible calcium mobilization from calcium oxalate crystals in seed protein bodies. J.N.A. Lott, E. Spitzer, M. White, and C. Vollmer, Department of Biology, McMaster University.

6. A clearing procedure for lipid-storing embryos of Acer saccharum. C.Y, Shih, Carol A. Peterson, and E.B. Dumbroff, Biology Department, University of Waterloo.

7. Effect of light intensity on lentil seedling morphology. Constance Nozzolillo, Biology Department, University of Ottawa.

8. Enzyme activity in dwarf and normal cultivars of pea. J. Ross, Scarborough College, University of Toronto.

12:20-1:50 Lunch and Posters, Room H-403B; Greenhouse

1:50-3:30 Third session

9. Scanning electron microscope study of leaf characteristics of pine and poplar. A. Eastham and D. Ormrod, University of Guelph, and T. Blake, University of Toronto.

10. The role of comparative developmental studies in understanding evolutionary changes: an example. A.D. MacDonald, Department of Biology, Lakehead University.

11. Aspects of plant development from the 13th International Botanical Congress, Sydney, Australia. U. Posluszny, University of Guelph; N. Dengler, University of Toronto; C. Peterson, University of Waterloo.

12. False images in EM studies of witch-hazel (family Haemorrhagidaceae). G. Host and Jack O. Lantern, Institute for Phantasmagorical Research, Goblin, Ontario.

3:30 Refreshments, Room H-403B

Are cell proliferation and cell growth regulated by a single control system?

D. Davidson. Biology Department, McMaster University, HAMILTON.

Roots of Pisum sativum were treated with 0.1% caffeine for 2 hr. and 1 hr. later some roots were also treated with 0.01% colchicine for 1 hr. Binucleate cells, induced by caffeine, were seen in division 16 hr. after the end of treatment: their mitotic index increased from 1, at 16 hr., to 8.2 at 20 hr. In roots also treated with colchicine, M.I. of binucleate cells was 36.3 at 16 hr. and 33.3 at 20 hr. Colchicine, therefore, stimulated the binucleate cells to complete a cell cycle and divide. The mean area of binucleate cells in mitosis, however, was only $\sim$ 1.5 times that of diploid, mononucleate, mitotic cells; thus, colchicine stimulates progress through interphase but does not stimulate the binucleate cells to double in area over one cell cycle. It is concluded that: 1) an external stimulus can increase the rate of progress through interphase without inducing an absolutely parallel increase in rate of cell growth; and 2) rate of progress through the cell cycle and rate of cell growth are not regulated by one mechanism, i.e. these parameters of cell growth are not under coordinate control. At least two control mechanisms must be involved and these are capable of responding independently to external stimuli.

Differences in Mean Doubling Time on Adjacent Rows of Cells. D.Davidson. Biology Department, McMaster University, Hamilton. Ont.

The colchicine accummulation method was used to determine mean doubling time, T_D, in epidermal and cortical rows of cells in roots of Zea mays. T_D was 7.34 hr in epidermal cells and 9.41hr in cortical cells. This is a striking difference between columns of cells that lie next to one another. A parallel difference is also seen in mean cell length: it is 11.7 $\pm$ 2.9µm in cortical cells and 9.6 $\pm$ 3.3µm in epidermal cells. An essential aspect of this study is that the cells scored occupied the same zone of the root, i.e. comparisons were made between cells in the same area of longitudinal sections. The results suggest that the relationship:

relative rate of change of mean cell length = local relative extension rate - local relative division rate

differs over a region of the root occupied by columns 45 cells long. Though a single control mechanism may regulate cell extension growth in adjacent columns of cells, the evidence shows that rates of cell proliferation are regulated independently in epidermal and cortical rows of cells.

(3)

Asymmetry of Mitosis: Are the Unequal Divisions Functionally significant?

S.W. Armstrong and D. Davidson. Biology Department, McMaster University. HAMILTON.

Nuclear division is accepted as producing two genetically identical nuclei. Cytokinesis, on the other hand is often asymmetrical and produces two daughter cells of different sizes. Data will be presented to show that asymmetry of cytokinesis is usual, and not an exceptional event. In roots of Zea mays we have found that, in cortical cell lineages, only 1% of sister cells have the same length. For pairs of sister cells sampled at all stages of the cell cycle, the average ratio of sister cell lengths, longer to shorter (L/S) is 1.2:1. For pairs of G_1 cells the average ratio is 1.21:1 and for pairs of sister cells with at least one cell in mitosis the ratio is 1.18:1. For triplets, where one cell is in mitosis and one cell has divided, the ratio is 1.2:1. Thus, the difference in length of sister cells is maintained throughout the cell cycle. We have also studied the polarity of this asymmetry, as revealed by the ratio of apical:basal cell length (where the cell closer to the root apex is defined as being the apical cell of a pair and the cell closer to the base of the root is basal; A and B). The A/B length ratio is not consistent either within one column of cells or between adjacent columns i.e. the A/B ratio is sometimes > 1 and sometimes < 1, e.g. 1.09:1 and 0.91:1 in adjacent columns of cells. This heterogeneity within a root suggests that: 1) the polarity of cytokinesis is not regulated to form a uniform pattern and, 2) spindle location within a cell can vary. It appears that groups of cells are acting, at least to some extent, undependent of each other. How the asymmetry of division relates to the oversee pattern of root growth will be reviewed.

The ultrastructure of cell and nucleomorph division in the alga Cryptomonas.
L.J. McKerracher and S.P.Gibbs, York University, Toronto and McGill University, Montreal.

Cell division of Cryptomonas sp. was investigated, with emphasis on the behavior of the nucleomorph (NM). The NM has been postulated to be a vestigal nucleus of a eukaryotic endosymbiont. The Golgi apparatus and flagella replicate during preprophase prior to NM division. NM division is not mediated by microtubules and is probably accomplished by an invagination of the NM envelope. Colchicine does not prevent NM division. The NMs move apart and following chloroplast division each daughter NM is associated with a chloroplast in a sac of chloroplast ER. Cytoplasmic microtubules from the flagellar region extend to the nucleus which migrates anteriorly. Gaps appear in the nuclear envelope (NE) and microtubules enter as the chromatin condenses. the two NMs are located near the spindle poles. Chromatin which is arranged at the equator at metaphase separates into two masses at anaphase. Some chromatin lags along the remnants of NE at the sides of the spindle. During reformation of the NE at telophase, the chromatin is irregularily shaped which is probably a result of the observed lag at anaphase. Cytokinesis is by longitudinal constriction.

Studies of Possible Calcium Mobilization from Calcium Oxalate Crystals in Seed Protein Bodies: John N.A. Lott, E. Spitzer, M. White and C. Vollmer, Department of Biology, McMaster University, Hamilton and Department of Botany, U.B.C., Vancouver.

Mobilization of calcium from endosperm into developing seedlings of several umbelliferous plants including carrot (Daucus carota, L. cv Imperator 408), wild carrot (Daucus carota L.), caraway (Carum carvi L.), anise (Pimpinella anisum L.), dill (Anethum graveolens L.), celery (Apium graveolens L. cv Tall Utah), fennel (Foeniculum vulgare Mill), parsnip (Pastinaca sativa L. cv Hollow Crown), parsley (Petroselinum sativum L. cv Moss Curled) and chervil (Anthriscus cerefolium L. cv Curled) was investigated with emphasis on carrot and celery. Calcium determinations using atomic absorption spectrometry of carrot and celery embryos obtained from dry mericarps and carrot and celery seedlings collected when the pericarp + testa + endosperm remains fell off the seedlings, revealed that some calcium uptake occurred in carrot and possibly celery. That some of the calcium obtained by the seedlings came from the calcium oxalate crystals is possible but the calcium could also have originated from other calcium sources within the endosperm. Polarized light studies of endosperm remains from carrot, celery and the other members of the family Umbelliferae investigated, revealed that large numbers of calcium oxalate crystals were present in the endosperm remains after separation from the seedlings. Light and electron microscopic studies of druse crystals in embryos of Eucalyptus erythrocorys and E. maculata indicate that these crystals are retained during germination and early seedling growth. The results of our studies indicate that calcium oxalate crystals are not used extensively as a calcium source during germination and early seedling growth.

6)

A Clearing Procedure for Lipid-storing Embryos of *Acer saccharum*

Ching-Yu Shih, Carol A. Peterson and E. B. Dumbroff
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Abstract. Clearing techniques have been used extensively for the study of vascular anatomy, sclereid distribution, detection of fungi in plants and the development of early embryos within the ovule, but a clearing procedure for the study of mature embryos, especially those with high percentage of lipids in their reserve food, has not been developed. Conventional clearing techniques do not give satisfactory results for lipid storing embryos due to their high lipid content. Embryos of *Acer saccharum* store food in the form of lipid, starch, protein and a material that stains positively with toluidine blue O (possibly phytin). In our clearing procedure, the embryos are decolorized and fixed in 1:1 mixture of glacial acetic acid and absolute ethanol. Gentle agitation for 48h in excess petroleum ether is used to facilitate lipid extration. The remaining lipids are removed by successive extractions with chloroform and acetone. Protein are removed by immersing the embryos in 4% sodium hydroxide at 60° for 3 days and then in saturated chloral hydrate at 60° for 2 days. The embryos then become transparent and fragile. They may be kept in chloral hydrate at room temperature for long period of time or, after washing with water, they can be stained with basic fuchsin and aniline blue to detect xylem and phloem respectively.

Enzyme activities in dwarf and normal cultivars of pea. J. Ross and G.F. Israelstam, Scarborough College, University of Toronto.

Using fluorometric techniques, the activities of the enzymes alcohol dehydrogenase and glucose-6-phosphate dehydrogenase were determined in dwarf and normal cultivars of pea. Seeds were separated into cotyledon and axis so that levels of these enzymes could be examined in each. In addition the effect of gibberellic acid (GA_3) on these activities was studied. Finally, the influence of the axis on enzyme activity in the cotyledon was examined by removing the axis and allowing the cotyledons to incubate alone.

In general, enzyme levels were greater in the normal than in the dwarf and in intact cotyledons as opposed to those which had their axis removed. Effects of GA_3 were minimal and seldom significant.

Scanning Electron Microscope Study of Leaf Characteristics of Pine and Poplar

A. Eastham and D. Ormrod, University of Guelph
and T. Blake, University of Toronto

Leaves of two pine species differing in sensitivity to sulphur dioxide (SO_2) and of one poplar species, were studied to compare leaf surface and stomatal features and to determine the effects of exposure to air pollutants. Three-year old seedlings of Pinus nigra and Pinus strobus and plants from hardwood cuttings of Populus nigra 'Italica' were exposed to SO_2, nitrogen dioxide (NO_2) or SO_2 + NO_2. Leaf samples were taken over a three week period following a six hour exposure. The samples were air dried, freeze dried, or fixed in glutaraldehyde and osmium. Prepared samples were mounted on stubs, coated with gold palladium, and viewed on a scanning electron microscope (S.E.M.).

A second study with poplar involved four clones previously rated for wood production on the basis of water usage and transpiration rate. Populus maximowiczii (Max 4) and Populus alba (A-499) are highly efficient wood producers on a per unit water basis, although Max 4 has a high transpiration rate and A-499 a low transpiration rate compared with other efficient wood producers. In contrast, N-80, a Populus deltoides x nigra clone, and Populus maximowiczii (Max 13) are low in wood production efficiency. N-80 has a high rate of transpiration, while Max 13 has a relatively low rate of transpiration. Leaf samples were air-dried, mounted and coated for S.E.M. viewing. Leaf surface features, and position, size and number of stomates were used to help explain transpiration and water use efficiency in wood production.

ALASTAIR D. MACDONALD. Department of Biology,
Lakehead University, Thunder Bay, Ontario, P7B 5E1.

The role of comparative developmental studies in understanding evolutionary changes: an example.

A study of the ontogeny of the female inflorescences and flowers of the Myricaceae permits one to identify which ontogenetic modifications most likely provide the mechanism whereby morphological differences arise. The composition of the fruit wall of three distinct morphological categories of myricaceous fruits will be reviewed. The ontogenetic basis for each category will be described to show how the inflorescence axis, including bracteate appendages, become incorporated into the gynoecial wall and consequently the fruit wall. One may infer from these data, as a working hypothesis, that fleshy and wax-secreting papillae, which cover the fruit, may be homologized to inflorescence bracts. This hypothesis was derived in the following manner: 1. There exists a potential for inflorescence bracts to be functionally associated with the gynoecium; 2. A structural association of bracts and gynoecium is brought about by zonal growth or zonal growth coupled with meristem extension and meristem incorporation which results in the gynoecium being comprised mainly of inflorescence axial tissue; 3. A reorganization of the developmental pattern, resulting in differentiation of papillae rather than bracts, and an elaboration of the number of papillae formed, results in the fruit wall being covered by papillae. Observations of the development of the fruit wall of Myrica pensylvanica and of the flower and fruit of Myrica gale and Comptonia peregrina illustrate these mechanisms of morphological change.

9/30/81

MINERAL RESERVES IN RICINUS SEEDS

John N.A. Lott, John Greenwood and Cathy M. Vollmer, Department of Biology, McMaster University, Hamilton and Department of Biology, University of Calgary, Calgary.

Mineral reserves in seeds of Ricinus communis L., cultivars Zanzibarensis and Hale, were studied. Elements present in globoid crystals in protein bodies were studied with energy dispersive x-ray (EDX) analysis. Prior to EDX analysis of globoid crystals, selected regions (cotyledon, radicle, hypocotyl, endosperm) either were glutaraldehyde fixed, dehydrated, embedded and sectioned or were prepared as freeze-dried powders. Quantitative methods, including atomic absorption spectrometry, were used to measure the amounts of K, Mg, P and Ca in selected tissues. Globoid crystals in endosperm normally contained P, K, and Mg although rarely some Ca was present. Globoid crystals in mesophyll or provascular cells of cotyledons contained P, K and Mg and rarely traces of Ca or Fe. In radicle and hypocotyl regions the ground meristem cells contained P, K and Mg in globoid crystals whereas in provascular cells some Ca was present in the globoid crystals along with P, K and Mg. Quantitative determinations showed that there was much less Ca than P, K or Mg in endosperm or embryo tissues on a weight basis. Most of the Ca present in castor bean seeds is in the testa where calcium oxalate crystals occur. Care must be taken not to contaminate endosperm samples with the crystal containing innermost testa region which adheres to the endosperm surface.

Abstract for P.O.W Poster

DEVELOPMENT OF LEAF DIMORPHISM IN SELAGINELLA MARTENSII

Nancy G. Dengler Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 1A1

The anisophyllous shoots of Selaginella martensii are characterized by opposite pairs of dorsal and ventral leaves which differ in both size and form. This study was undertaken to determine the developmental mechanisms which result in the dimorphism of these leaves which are produced more or less simultaneously on the same shoot apex, and to test the hypothesis that the small dorsal leaf is an arrested form of the larger ventral leaf. Observations of whole cleared leaves at successive stages of development, serial cross and longitudinal sections of shoot tips and Feulgen-stained dissected leaves reveal that: 1) while the pattern of cell divisions associated with leaf initiation is similar, the primordium of the ventral leaf is larger and has a greater tangential extent, 2) expansion of each leaf type occurs at the same rate, but is of longer duration in the ventral leaf, and 3) both cell division and cell enlargement occur over a longer time period in the ventral leaves, indicating that the smaller size of the dorsal leaves is the result of precocious maturation. Quantitative histological measurements document a strong basipetal pattern of tissue maturation in both leaf types, and the early development of distinctive histological features. The presence of distinguishing morphological and anatomical features shortly after initiation indicates that the small size of the dorsal leaf is not the result of developmental arrest.

(12)

False Images in EM Studies of Witch-Hazel (Family Haemorrhagidaceae).

G. Host and Lantern, Jacko. Institute for Phantasmagorical Research. Goblin, Ontario.

It had previously been reported (Stein and Frank, 1978) that electron microscopic studies of witch-hazel yielded false images of the hooded section of the leaf-cape that surrounds the broom. Our studies have concentrated on this hooded structure, the een, which varies in structure. The een is used as a blood storage vessel by bats; in some cases it is filled with blood but in other cases the een is hollow. We are interested in the hollow een. Pictures taken of the een, particularly in the middle of the lunar cycle give false images; these false images must result from an alteration of the flux of electrons inside our microscope. We are currently haunted by our inability to explain why our clean vacuum system produces an unusual electroflux at this time of year. (Research supported by grants from the Nocturnal Scary Events Research Council and the British Vampire Campaign of Drag-Ula, Bohemia.).